

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as shown:

Please delete the paragraph on page 5, line 32, to page 6, line 9, and replace it with the following paragraph:

In normal *S. cerevisiae* (budding yeast) a cells, the α -factor binds the G protein-coupled membrane receptor STE2. The G protein dissociates into the G_α and $G_{\beta\gamma}$ subunits, and the $G_{\beta\gamma}$ binds an unidentified effector, which in turn activates a number of genes. STE20, a kinase, activates STE5, a protein of unknown function. STE5 activates STE11 kinase, which stimulates STE7 kinase, which induces the KSS1 and/or FUS3 kinases. These switch on expression of the transcription factor STE12. STE12 stimulates expression of a wide variety of genes involved in mating, including FUS1 (cell fusion), FAR1 (cell-cycle arrest), STE2 (the receptor), MFA1 (the pheromone), SST2 (recovery), KAR3 (nuclear fusion) and STE6 (pheromone secretion). Other genes activated by the pathway are CHS1, AG α 1, and KAR3. The multiply tandem sequence TGAAACA (**SEQ ID NO: 128**) has been recognized as a "pheromone response element" found in the 5'-flanking regions of many of the genes of this pathway.

Please delete the paragraph on page 23, lines16-28, and replace it with the following paragraph:

Figure 11. This Figure schematically describes three hybrids of GPA1 and Gas. The – LLLLGAGES- sequence (**SEQ ID NO: 120**) demarcated in GPA1 directly follows the non-conserved N-terminal domain of the protein (**SEQ ID NO: 135**). The longer sequence demarcated in GPA1 encodes the "switch region" believed to be involved in the conformational change that occurs with nucleotide exchange upon receptor activation. 41-Gas is comprised of the N-terminal 41 amino acids of GPA1 linked to Gas sequence from which the native N-terminal sequence has been deleted. SGS denotes a molecule comprised of the switch region residues of GPA1 replacing those of Gas. GPA₄₁-SGS includes both the N-terminal and switch region sequences of GPA1 inserted into Gas. (See Table 6 for the exact sequence junctions used to construct

these hybrid proteins).

Please delete the paragraph on page 46, lines 23-37, and replace it with the following paragraph:

In Table 5, part A, the amino terminal 66 residues of GPA1 are aligned with the cognate domains of human G α s, G α i2, G α i3 and G α 16. In part B, we present alignment of the amino-terminal 66 residues of GPA1₄₁-G α chimeras. In the GPA1₄₁-G α hybrids, the amino terminal 41 residues (derived from GPA1) are identical, end with the sequence –LEKQRDKNE- (**SEQ ID NO: 121**) and are underlined for emphasis. All residues following the glutamate (E) residue at position 41 are contributed by the human G α subunits, including the consensus nucleotide binding motif –GxGxxG- (**SEQ ID NO: 122**). Periods in the sequences indicate gaps that have been introduced to maximize alignments in this region. Codon bias is mammalian. For alignments of the entire coding regions of GPA1 with G α s, G α i, and G α O, G α q and G α z, see Dietzel and Kurjan (1987) and Lambright, et al. (1994). Additional sequence information is provided by Mattera, et al. (1986), Bray, et al. (1986) and Bray, et al. (1987).

Please delete the paragraph on page 48, lines 16-37, and replace it with the following paragraph:

As indicated above, there is little if any sequence homology shared among the amino termini of G α subunits. The amino terminal domains of G α subunits that precede the first β -sheet (containing the sequence motif –LLLLGAGESG (**SEQ ID NO: 123**); see Noel, et al. (1993) for the numbering of the structural elements of G α subunits) vary in length from 41 amino acids (GPA1) to 31 amino acids (G α t). Most G α subunits share the consensus sequence for the addition of myristic acid at their amino termini (MGxxxS – (**SEQ ID NO: 124**)), although not all G α subunits that contain this motif have myristic acid covalently associated with the glycine at position 2 (speigel, et al. 1991). The role of this post-translational modification has been inferred from studies in which the activity of mutant G α subunits from which the consensus sequence for myristoylation has been added or deleted has been assayed (Mumby, et al. 1990; Linder, et al. 1991; Gallego,

et al. 1992). These studies suggest two roles for N-terminal myristoylation. First, the presence of amino-terminal myristic acid has in some cases been shown to be required for association of G α subunits with the membrane, and second, this modification has been demonstrated to play a role in modulating the association of G α subunits with G $\beta\gamma$ complexes. The role of myristoylation of the GPA1 gene product is, at present, unknown.

Please delete the paragraph on page 55, line 28, to page 56, line 4, and replace it with the following paragraph:

Several predictive algorithms indicate that the amino terminal domain up to the highly conserved sequence motif –LLLLGAGESG- (**SEQ ID NO: 129**) (the first L in this motif is residue 43 in GPA1) forms a helical structure with amphipathic character. Assuming that a heptahelical repeat unit, the following hybrids between GPA1 and Gas can be used to define the number of helical repeats in this motif necessary for hybrid function:

GPA1-7/Gas8-394

GPA1-14/Gas15-394

GPA1-21/Gas22-394

GPA1-28/Gas29-394

GPA1-35/Gas36-394

GPA1-42/Gas43-394

Please delete the paragraph on page 56, lines 19-21, and replace it with the following paragraph:

The gap that is introduced between residues 9 and 10 in the Gas sequence is to preserve the alignment of the –LLLLGAGE- (**SEQ ID NO: 130**) sequence motif.

Please delete the paragraph on page 57, line 29, to page 58, line 15, and replace it with the following paragraph:

GPA_{ID} and GPA_{LW} hybrids. The regions of high homology among Gα subunits that have been identified by sequence alignment are interspersed throughout the molecule. The G1 region containing the highly conserved -GSGESGDST- (**SEQ ID NO: 125**) motif is followed immediately by a region of very low sequence conservation, the "il" or insert 1 region. Both sequence and length vary considerably among the il regions of the Gα subunits. By aligning the sequences of Gα subunits, the conserved regions bounding the il region were identified and two additional classes of GPA1-Gα hybrids were constructed. The GPA_{ID} hybrids encode the amino terminal 102 residues of GPA1 (up to the sequence -QARKLGIQ- (**SEQ ID NO: 126**)) fused in frame to mammalian Gα subunits, while the GPA_{LW} hybrids encode the amino terminal 244 residues of GPA1 (up to the sequence -LIHEDIAKA- (**SEQ ID NO: 127**) in GPA1). The reason for constructing the GPA_{ID} and GPA_{LW} hybrids was to test the hypothesis that the il region of GPA1 is required for mediating the interaction of GPA1 with yeast Gβγ subunits, for the stable expression of the hybrid molecules, or for function of the hybrid molecules. The GPA_{ID} hybrids contain the amino terminal domain of GPA1 fused to the il domain of mammalian subunits, and therefore do not contain the GPA1 il region, while the GPA_{LW} hybrids contain the amino terminal 244 residues of GPA1 including the entire il region (as defined by sequence alignments). Hybrids of both GPA_{ID} and GPA_{LW} classes were constructed for Gα_S, Gα_{i2}, Gα_{i3}, Gα_o, and Gα₁₆; none of these hybrids complemented the *gpa1* growth arrest phenotype.

Please delete the paragraph on page 59, lines 11-34, and replace it with the following paragraph:

Gα_S Hybrids. There is evidence that the "switch region" encoded by residues 171-237 of Gα transducin (using the numbering of Noel et al (1993)) also plays a role in Gβγ coupling. First, the G226A mutation in Gα_S (Miller et al. 1988) prevents the GTP-induced conformational change that occurs with exchange of GDP for GTP upon receptor activation by ligand. This residue maps to the highly conserved sequence –

Please delete the paragraph on page 93, line 37, to page 94, line 23, and replace it with the following paragraph:

α-factor
oligo: TGG CAT TGG TTG CAG CTA AAA CCT GGC CAG CCT ATG
TAC
encodes Trp His Trp Leu Gln Leu Lys Pro Gly Gln Pro Met Tyr
(DNA, SEQ ID NO: 91, AAS, SEQ ID NO: 92)

M1 TGG CAT TGG TTG TCC TTG TCG CCC GGG CAG CCT ATG
TAC
encodes Trp His Trp Leu Ser Leu Ser Pro Gly Gln Pro Met Tyr
(DNA, SEQ ID NO: 93, AAS, SEQ ID NO: 94)

M2 TGG CAT TGG TTG **TCC CTG GAC GCT GGC** CAG CCT ATG
TAC
encodes Trp His Trp Leu Ser Leu Asp Ala Gly Gln Pro Met Tyr
(DNA, SEQ ID NO: 95, AAS, SEQ ID NO: 96)

M3 TGG CAT TGG TTG **ACC TTG ATG GCC GGG** CAG CCT ATG
TAC
encodes Trp His Trp Leu Thr Leu Met Ala Gly Gln Pro Met
Tyr
(DNA, SEQ ID NO: 97, AAS, SEQ ID NO: 98)

M4 TGG CAT TGG TTG **CAG CTG TCG GCG GGC** CAG CCT ATG
TAC
encodes Trp His Trp Leu Gln Leu Ser Ala Gly Gln Pro Met
Tyr
(DNA, SEQ ID NO: 99, AAS, SEQ ID NO: 100)

M5 TGG CAT TGG TTG **AGG TTG CAG TCC GGC** CAG CCT ATG
TAC
encodes Trp His Trp Leu Arg Leu Gln Ser Gly Gln Pro Met
Tyr
(DNA, SEQ ID NO: 101, AAS, SEQ ID NO: 102)

M6 TGG CAT TGG TTG **CGC TTG TCC GCC GGG** CAG CCT ATG
TAC
encodes Trp His Trp Leu Arg Leu Ser Ala Gly Gln Pro Met
Tyr
(DNA, SEQ ID NO: 103, AAS, SEQ ID NO: 104)

M7 TGG CAT TGG TTG **TCG CTC GTC CCG GGG** CAG CCT ATG
TAC

encodes Trp His Trp Leu Ser Leu Val Pro Gly Gln Pro Met
Tyr

(DNA, SEQ ID NO: 105, AAS, SEQ ID NO: 106)

M8 TGG CAT TGG TTG **TCC CTG TAC CCC GGG** CAG CCT ATG
TAC

encodes Trp His Trp Leu Ser Leu Tyr Pro Gly Gln Pro Met
Tyr

(DNA, SEQ ID NO: 107, AAS, SEQ ID NO: 108)

M9 TGG CAT TGG TTG **CGG CTG CAG CCC GGG** CAG CCT ATG
TAC

encodes Trp His Trp Leu Arg Leu Gln Pro Gly Gln Pro Met
Tyr

(DNA, SEQ ID NO: 109, AAS, SEQ ID NO: 110)

Please delete the paragraph on page 95, lines 4-27, and replace it with the following paragraph:

A semi-random α -factor library was obtained through synthesis of mutagenized α -factor oligonucleotides such that 1 in 10,000 peptide products were expected to be genuine α -factor. The mutagenesis was accomplished with doped synthesis of the oligonucleotides: each nucleotide was made approximately 68% accurate by synthesizing the following two oligos:

5' CTGGATGCGA **AGACT**CAGCT (20 mer) (oligo060) (SEQ ID NO:44)

where the FokI site is underlined and the BbsI site is emboldened.

5' CGGATGATCA gta cat tgg ttg gcc agg ttt tag ctg caa cca atg cca AGC TGA
GTC TTC GCA TCC AG (69 mer) (oligo074) (SEQ ID NO: 45)

where the BclI site is italicized, the FokI site is underlined, the BbsI site is emboldened. The lower case letters indicate a mixture of 67% of that nucleotide and 11% of each of the other three nucleotides (e.g. t indicates 67% T and 11% A, 11% C, and 11% G). Note that digestion of the double-stranded oligonucleotide by FokI or BbsI will yield an identical 5' end that is compatible with HindIII ends.

Oligos 060 and 074 will form the following double-stranded molecule when annealed:

5' CTGGATGCGAAGACTCAGC T **(SEQ ID NO: 44)**

3' GACCTACGCTTCTGAGTCGA acc gta acc aac gtc gat ttt gga ccg gtt ggt tac atg
ACTAGTAGGC 5' **(SEQ ID NO: 45)**

Please delete the paragraph on page 98, line 27, to page 99, line 15, and replace it with the following paragraph:

Random oligonucleotides to be expressed by the expression plasmid CADUS 1215 will encode tridecapeptides constructed as 5' CGTGAAGCTTAAGCGTGAGGCAGAAGCT(NNK)₁₃TGATCATCCG, (SEQ ID NO:6) where N is any nucleotide, K is either T or G at a ratio of 40:60 (see Proc Natl Acad Sci 87:6378, 1990; *ibid* 89:5393, 1992), and the AflII and BclI sites are underlined. This oligonucleotide is designed such that: the AflII and BclI sites permit inserting the oligos into the AflII and BglII site of CADUS 1215 (see Figure 4); the HindIII site just 5' to the AflII site in the 5' end of the oligo allows future flexibility with cloning of the oligos; the virtual repeat of GAGGCT **(SEQ ID NO: 131)** and the GAGA **(SEQ ID NO: 132)** repeats which are present in the wild-type sequence and which can form triple helixes are changed without altering the encoded amino acids. The random oligonucleotides described above will actually be constructed from the following two oligos:

5' CGTGAAGCTTAAGCGTGAGGCAGAAGCT (SEQ ID NO: 26) and

5' CGGATGATCA(MNN)₁₃AGCTTCTG (SEQ ID NO: 27),

where M is either A or C at a ratio of 40:60. The oligos will be annealed with one another and repetitively filled in, denatured, and reannealed (Kay et al, Gene, 1993). The double-stranded product will be cut with AflII and BclI and ligated into the AflII-and

BglII-digested CADUS 1215. The BglII/BclI joint will create a TGA stop codon for termination of translation of the randomers (Figure 4). Because of the TA content of the Afl overhang, the oligos will be ligated to the AflII-and BglII-digested pADC-MF α at 4°C.

Please delete the paragraph on page 100, line 32, to page 101, line 12, and replace it with the following paragraph:

A single plasmid (corresponding to a bacterial colony) was obtained from each of the ten isolates, and reintroduced into CY2012. Eight of the ten plasmids passed the test of retaining the ability to confer the His⁺ phenotype on CY2012 (the two that failed correspond to the two isolates that were mentioned above, suggesting that these isolates contain at least one "irrelevant" plasmid). Sequencing of the randomized insert in the eight plasmids of interest revealed that four contain the sequence:

TAT GCT CTG TTT GTT CAT TTT TTT GAT ATT CCG (SEQ ID NO: 52)

Tyr Ala Leu Phe Val His Phe Phe Asp Ile Pro, (SEQ ID NO: 53)

two contain the sequence:

TTT AAG GGT CAG GTG CGT TTT GTG GTT CTT GCT (SEQ ID NO: 54)

Phe Lys Gly Gln Val Arg Phe Val Val Leu Ala, (SEQ ID NO: 55)

and two contain the sequence:

CTT ATG TCT CCG TCT TTT TTT TTT TTG CCT GCG (SEQ ID NO: 56)

Leu Met Ser Pro Ser Phe Phe Phe Leu Pro Ala (SEQ ID NO: 57)

Clearly, these sequences encode novel peptides, as the native α -factor sequence differs considerably:

Tyr Ile Ile Lys Gly Val Phe Trp Asp Pro Ala **(SEQ ID NO: 133)**.

Please delete the paragraph on page 112, line 12, to page 113, line 8, and replace it with the following paragraph:

A hybrid gene encoding the prepro-region of human POMC (accession # K02406; Takahashi, H., et al (1983) Nucleic Acids Research 11:6847-6858) and the coding region of a single repeat of mature α -factor will be constructed in the following fashion. The prepro-region of human POMC will be amplified with an HindIII site at the 5' end and a BbsI site at the 3' end using VENT polymerase and the following primers: 5' GGGAAGCTT ***ATGCCGAGATCGTGCTGCCAGCCGC*** 3' (SEQ ID NO:30) (HindIII site is underlined and initiation codon is italic bold) and antisense 5' GGGGAAGACTTCTGCCCTGCGCCGCTGCTGCC 3' (SEQ ID NO:31) (BbsI recognition is underlined), leaving the amino acid sequence -SSGAGQKR—**(SEQ ID NO: 119)** at the 3' end with a BbsI site leaving an overhang at the -KR- dibasic cleavage sequence. The coding region of α -factor will be amplified from Cadus 1219 with a BbsI site at the 5' end and a BglII site at the 3' end using the primers 5' GGGGAAGACCCGCAGGAGGCAGAAGCTT GGTTCAG 3' (SEQ ID NO:32) (BbsI site is underlined) and 5' GGGAGATCT**TC**AGTACATTGGTTGGCC 3' (SEQ ID NO:33) (BglII site is underlined, termination codon is bold) The PCR fragment encoding the pre-pro segment of POMC is restricted with HindIII and BbsI and gel purified, the PCR fragment encoding α -factor is cut with BbsI and BglII and gel purified, and Cadus 1215 is cut with BglII and partially with HindIII and the HindIII-BglII restricted vector containing the pAlter polylinker sequences is gel purified. Three-part ligation of the two PCR products with HindIII and BglII digested Cadus 1215 will yield a hybrid POMC/ α -factor gene in which the first 104 amino acids residues are from POMC and the remaining 17 are from α -factor. The structure of this hybrid gene around the PC1 cleavage site is: - RNSSSSGSSGAGOKREAEAWHWLQLKPGQPMY* (SEQ ID NO:34) where residues donated by POMC are underlined, the dibasic cleavage site is

underlined bold, and the sequence of mature α -factor is in italics. The tetrapeptide - EAEA-(**SEQ ID NO: 134**) juxtaposed between the dibasic cleavage site and the amino-terminal tryptophan of mature α -factor should be removed by the dipeptidyl aminopeptidase activity of ste13p.

Please delete the paragraphs on page 123, line 13, to page 125, line 16, and replace them with the following paragraphs:

Site-directed mutagenesis of *GPA1* (Cadus 1075, 1121 and 1122). A 1.9 kb EcoRI fragment containing the entire *GPA1* coding region and 200 nucleotides from the 5' untranslated region was cloned into EcoRI cut, phosphatase-treated pALTER-1 (Promega) and transformed by electroporation (Biorad Gene Pulser) into DH5 α F' bacteria to yield Cadus 1075. Recombinant phagemids were rescued with M13KO7 helper phage and single stranded recombinant DNA was extracted and purified according to the manufacturer's specifications. A new NcoI site was introduced at the initiator methionine of *GPA1* by oligonucleotide directed mutagenesis using the synthetic oligonucleotide:

5' GATATATTAAGGTAGGAAACCATGGGGTGTACAGTGAG 3' (**SEQ ID NO: 66**).

Positive clones were selected in ampicillin and several independent clones were sequenced in both directions across the new NcoI site at +1. Two clones containing the correct sequences were retained as Cadus 1121 and 1122.

Construction of a *GPA1*-based expression vector (Cadus 1127).

The vector used for expression of full length and hybrid mammalian G α proteins in yeast, Cadus 1127, was constructed in the following manner. A 350 nucleotide fragment spanning the 3' untranslated region of *GPA1* was amplified with Taq polymerase (AmpliTaq; Perkin Elmer) using the oligonucleotide primers A (5' CGAGC-GCTCGAGGGAACGTATAATTAAAGTAGTG 3' (**SEQ ID NO: 67**)) and B (5' GCGCGGTACCAAGCTTC- AATTCGAGATAATACCC 3' (**SEQ ID NO: 68**)). The 350 nucleotide product was purified by gel electrophoresis using GeneClean II (Bio101) and was cloned directly into the pCRII vector by single nucleotide overlap TA cloning (Invitrogen). Recombinant clones were characterized by restriction enzyme mapping and by dideoxynucleotide sequencing. Recombinant clones contained a novel XhoI site

5' to the authentic *GPA1* sequence and a novel KpnI site 3' to the authentic *GPA1* sequence donated respectively by primer A and primer B.

The NotI and SacI sites in the polylinker of Cadus 1013 (pRS414) were removed by restriction with these enzymes followed by filling in with the Klenow fragment of DNA polymerase I and blunt end ligation to yield Cadus 1092. The 1.4 kb PstI - EcoRI 5' fragment of *GPA1* from YCplac111-*GPA1* containing the *GPA1* promoter and 5' untranslated region of *GPA1* was purified by gel electrophoresis using GeneClean (Bio101) and cloned into PstI - EcoRI restricted Cadus 1013 to yield Cadus 1087. The PCR amplified XhoI - KpnI fragment encoding the 3' untranslated region of *GPA1* was excised from Cadus 1089 and cloned into XhoI - KpnI restricted Cadus 1087 to yield Cadus 1092. The NotI and SacI sites in the polylinker of Cadus 1092 were removed by restriction with these enzymes, filling in with the Klenow fragment of DNA polymerase I, and blunt end ligation to yield Cadus 1110. The region of Cadus 1122 encoding the region of *GPA1* from the EcoRI site at -200 to +120 was amplified with Vent DNA polymerase (New England Biolabs, Beverly, MA) with the primers

5' CCCGAATCCACCAATTTCTTTACG 3' (SEQ ID NO: 69) and

5' GCGGCGTCGACGCGGCCGCGTAACAGT 3' (SEQ ID NO: 70).

The amplified product, bearing an EcoRI site at its 5' end and novel SacI, NotI and Sall sites at its 3' end was restricted with EcoRI and Sall, gel purified using GeneClean II (Bio101), and cloned into EcoRI and Sall restricted Cadus 1110 to yield Cadus 1127. The DNA sequence of the vector between the EcoRI site at -200 and the KpnI site at the 3' end of the 3' untranslated region was verified by restriction enzyme mapping and dideoxynucleotide DNA sequence analysis.

PCR amplification of *GPA*₄₁-Gα proteins and cloning into Cadus 1127.

cDNA clones encoding the human G alpha subunits Gas, *Gai2*, *Gai3*, and *S. cerevisiae GPA1* were amplified with Vent thermostable polymerase (New England Biolabs, Beverly, MA). The primer pairs used in the amplification are as follows:
GαS Primer 1: 5'CTGCTGGAGCTCCGCCTGCTGCTGCTGGGTGCTGGAG3' (SacI 5') (SEQ ID NO: 71)

Primer 2: 5'CTGCTGGTCGACGCGGCCGCGGGGGTTCCTTCTT
AGAAGCAGC3'(Sall 3') (SEQ ID NO: 72)

Primer 3: 5'GGGCTCGAGCCTTCTTAGAGCAGCTCGTAC3' (XhoI 3') **(SEQ ID NO: 73)**

Gai2 Primer 1: 5'CTGCTGGAGCTCAAGTTGCTGCTGTTGGGTGCTGGGG3'
(SacI 5') **(SEQ ID NO: 74)**

Primer 2:
5'CTGCTGGTCGACGCGGCCGCGCCCCTCAGAAGAGCCGCGGTCC3' (Sall 3') **(SEQ ID NO: 75)**

Primer 3: 5'GGGCTCGAGCCTCAGAAGAGGCCGCAGTC3' (XhoI 3') **(SEQ ID NO: 76)**

Gai3 Primer 1: 5'CTGCTGGAGCTCAAGCTGCTGCTACTCGGTGCTGGAG3' (SacI 5') **(SEQ ID NO: 77)**

Primer 2:
5'CTGCTGGTCGACGCGGCCGCCACTAACATCCATGCTTCTCAATAA
AGTC3' (Sall 3') **(SEQ ID NO: 78)**

Primer 3: 5'GGGCTCGAGCATGCTTCTCAATAAAGTCCAC3' (XhoI 3')
(SEQ ID NO: 79)

After amplification, products were purified by gel electrophoresis using GeneClean II (Bio101) and were cleaved with the appropriate restriction enzymes for cloning into Cadus 1127.

Please delete the paragraph on page 125, line 33, to page 126, line 27, and replace it with the following paragraph:

Construction of GPA_{Bam}-Gα Constructs. A novel BamHI site was introduced in frame into the GPA1 coding region by PCR amplification using Cadus 1179 (encoding a wildtype GPA1 allele with a novel NcoI site at the initiator methionine) as the template, VENT polymerase, and the following primers: Primer A = 5' GCATCCATCAATAATCCAG 3' **(SEQ ID NO: 80)** and Primer B = 5'

GAAACAATGGATCCACTTCTTAC 3' (**SEQ ID NO: 81**). The 1.1 kb PCR product was gel purified with GeneClean II (Bio101), restricted with NcoI and BamHI and cloned into NcoI-BamHI cut and phosphatased Cadus 1122 to yield Cadus 1605. The sequence of Cadus 1605 was verified by restriction analysis and dideoxy-sequencing of double-stranded templates. Recombinant GPA_{Bam}-Gα₁ hybrids of Gas, Gai2, and Gα16 were generated. Construction of Cadus 1855 encoding recombinant GPA_{Bam}-Gα₁₆ serves as a master example: construction of the other hybrids followed an analogous cloning strategy. The parental plasmid Cadus 1617, encoding native Gα16, was restricted with NcoI and BamHI, treated with shrimp alkaline phosphatase as per the manufacturer's specifications and the linearized vector was purified by gel electrophoresis. Cadus 1605 was restricted with NcoI and BamHI and the 1.1 kb fragment encoding the amino terminal 60% of GPA1 with a novel BamHI site at the 3' end was cloned into the NcoI- and BamHI-restricted Cadus 1617. The resulting plasmid encoding the GPA_{Bam}-Gα₁₆ hybrid was verified by restriction analysis and assayed in tester strains for an ability to couple to yeast Gβγ and thereby suppress the *gpa1* null phenotype. Two additional GPA_{Bam}-Gα₁ hybrids, GPA_{Bam}-Gas₁ and GPA_{Bam}-Gai2, described in this application were prepared in an analogous manner using Cadus1606 as the parental plasmid for the construction of the GPA_{Bam}-Gα₁₂ hybrid and Cadus 1181 as the parental plasmid for the construction of the GPA_{Bam}-Gα₁₈ hybrid.

Please delete Table 5 on pages 150-151, and replace it with the following table:

TABLE 5.

Sequence alignments of N-terminal regions of Gα subunits and N-terminal sequences of GPA₄₁-Gα hybrid proteins.

A. Alignment of GPA1 with Gα Subunits

GPA1

MGC.TVSTQTIGDESDPFLQNKRANDVIEQSLQLEKQRDKNEIKLLLLGAGESGKSTVL

KQLKLLHQ.....

(SEQ ID NO: 82)

GαS

MGCLGTS..KTEDQRNEEKAQREANKKIEKQLQKDKQVYRATHRLLLLGAGESGKSTIV
KQMRILHV.....

(SEQ ID NO: 83)

Gαi2

MGC.TVS.....AEDKAAAERSKMIDKNLREDGEKAAAREVKLLLLGAGESGKSTIVKQMK
IIHE.....

(SEQ ID NO: 84)

Gαi3

MGC.TVS.....AEDKAAVERSKMIDRNLRDGEKAAKEVKLLLLGAGESGKSTIVKQMK
IIHE.....

(SEQ ID NO: 85)

Gα16

MARSLTWRCCPWCLTEDEKAAARVDQEINRILLEQKKQDRGELKLLLLGPGESGKSTF
IKQMRIIHG.....

(SEQ ID NO: 86)

B. GPA₄₁-Gα Hybrids

GPA₄₁-GαS

MGC.TVSTQTIGDESDPFLQNKRANDVIEQSLQLEKQRDKNERKLLLLGAGESGKSTIV
KQMRILHV.....

(SEQ ID NO: 87)

GPA₄₁-Gαi2

MGC.TVSTQTIGDESDPFLQNKRANDVIEQSLQLEKQRDKNEVKLLLLGAGESGKSTIV
KQMKIIHE.....

(SEQ ID NO: 88)

GPA₄₁-Gαi3

MGC.TVSTQTIGDESDPFLQNKRANDVIEQSLQLEKQRDKNEVKLLLLGAGESGKSTIV
KQMKIIHE.....

(SEQ ID NO: 89)

GPA₄₁-Gα16

MGC.TVSTQTIGDESDPFLQNKRANDVIEQSLQLEKQRDKNELKLLLLGPGESGKSTFI

KQMRIIHG.....

(SEQ ID NO: 90)

Please delete Table 7 on page 153, and replace it with the following table:

TABLE 7

Gα Subunit Alignment - "Switch Region"

β2 β3 α2 β4

GPA1

RIDTTGITETEFNIGSSKFKVLDAGGQRSEKRWIHC FEGITAVLFVLAMSEYDQ
MLFEDER

(SEQ ID NO: 111)

GαS .VL.S..F..K.QNDKVN.HMF.V....D.....Q..NDV..II..V.S.S.NMVIR..NQ

(SEQ ID NO: 112)

Gαi2 .VK....V..H.TFKDLH..MF.V.....V..II.CV.L.A..LV.ADE.M

(SEQ ID NO: 113)

Gαi3 .VK....V..H.TFKDLY..MF.V.....V..II.CV.L.D..LV.A...E

(SEQ ID NO: 114)

Gα0 .VK....V..H.TFKNLH.RLF.V.....DV..II.CN.L.G...V.H...T

(SEQ ID NO: 115)

Gα11 .VP....I.YP.DLENII..MV.....NV.SIM.LV.L....C.E.NNQ

(SEQ ID NO: 116)

Gα16 .MP....N.YC.SVQKTNL.IV.....N.I.LIYLASL.....V.V.SDN

(SEQ ID NO: 117)